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Cross-linking of starch with bifunctional precursors of nitroalkenes

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Abstract

Granular starch was cross-linked with 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (**1**), 1,3-di-*O*-pivaloyl-2-nitro-1,3-propanediol (**2**), 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**), 1,3-di-*O*-acetyl-*aci*-2-nitro-1,3-propanediol (**4**), 1,3-di-*O*-pivaloyl-*aci*-2-nitro-1,3-propanediol (**5**) and 1,6-di-*O*-acetyl-2,5-dinitro-1,6-hexanediol (**6**). The bifunctional precursors for the nitro-alkenes **1**, **2**, **3**, and **4** were readily synthesized in high yields from nitromethane, paraformaldehyde and acetic anhydride (**1**, **3**) or pivaloyl chloride (**2**, **4**), respectively. The reaction rate for the cross-linking was very high, and for **1** and **3**, the reaction reached completion within 1 h (at room temperature). The swelling capacities of the products obtained when starch was cross-linked with precursors for the nitroalkenes **1–4** and **6** were lower in comparison to epichlorohydrin cross-linked starch. These results indicate a high reaction efficiency at low degrees of substitution. Cross-linked 2-nitroalkyl starch ethers were synthesized in a one-pot synthesis by addition of **1** or **3** and 2-nitroalkyl acetates to granular suspensions of starch. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: Starch, cross-linking; Cross-linked 2-nitroalkyl starches

1. Introduction

The development of derivatization methods for polysaccharides with various reagents has included reactions with bifunctional compounds that lead to cross-linked polymers. Thus larger molecular

aggregates with different rheological behaviour, or insoluble products with a wide range of swelling characteristics, may be synthesized. The insoluble cross-linked polysaccharides have found several applications, e.g., in chromatography (gel filtration) [1]. Well known examples of these compounds are cross-linked dextrans (Sephadex) and agaroses (Sephacrose) [2].

In the starch industry the cross-linking of starch granules with bi- or polyfunctional reagents has led

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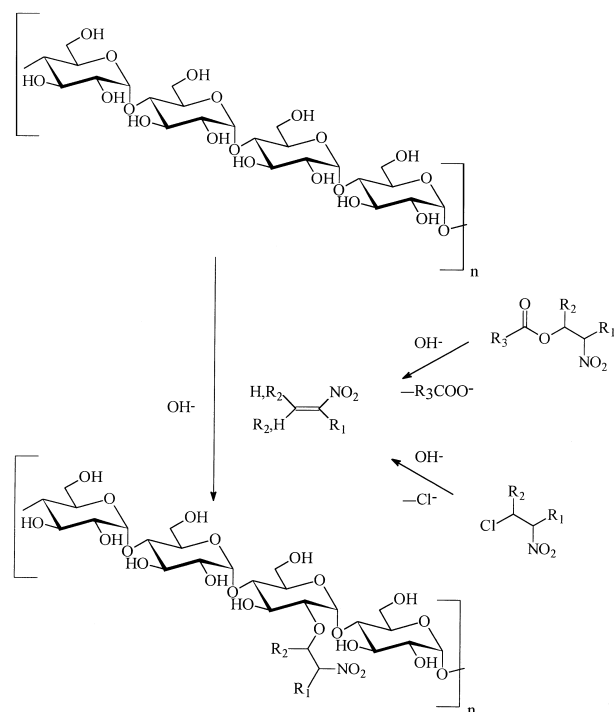
to a wide variety of commercial products in the food, textile and adhesives industries [3,4]. Not only are the cross-linked starches interesting compounds themselves, but they can also be subjected to other chemical modifications such as acetylation, hydroxypropylation or carboxymethylation. In this way products with widely different properties and applications have been synthesized.

Although a wide variety of cross-linking agents for starch and other polysaccharides have been described in the literature, only a few are of commercial importance. Examples are adipic anhydrides [5], phosphorus oxychloride [6], sodium trimetaphosphate [7] and epichlorohydrin [8]. The cross-linking of starch with these compounds can easily be achieved in aqueous starch suspensions under alkaline reaction conditions. Cross-linking of water soluble starches is less favourable for industrial applications. The cross-linked starch adipates and phosphates are used on a large scale in the food industry. A disadvantage of the distarch adipates and phosphates is the instability of the ester moieties under alkaline conditions.

Cross-linking of starch with epichlorohydrin leads to distarch glycerol. Due to the toxicity of epichlorohydrin, these cross-linked starches cannot be applied in the food sector. Distarch glycerol is highly resistant towards shear and owing to the ether linkages the epichlorohydrin cross-linked starch is stable under acidic and alkaline conditions. A slight disadvantage of the reaction is the relatively long reaction time required to achieve satisfactory degrees of conversion.

Recently, we developed (Scheme 1) a route to 2-nitroalkyl starch ethers by adding nitroalkenes, or precursors thereof, to aqueous starch suspensions [9–11]. High reaction rates were obtained in all cases for 2-nitroalkenes (or suitable precursors thereof) and the efficiency of the nitroalkylation was found to be especially high when 2-nitroalkenes were formed in situ from β -nitro-acyloxy-alkanes.

Here, we report the efficient cross-linking of granular starch with readily available bifunctional precursors of nitroalkenes. Similar to distarch glycerol these compounds are stable under alkaline and moderately acidic conditions owing to the ether linkages. Due to the excellent Michael acceptor capacities of nitroalkenes the rate of the cross-linking reaction is very high. The synthesis of cross-linked 2-nitroalkyl starches, which have very interesting application potential and are excellent



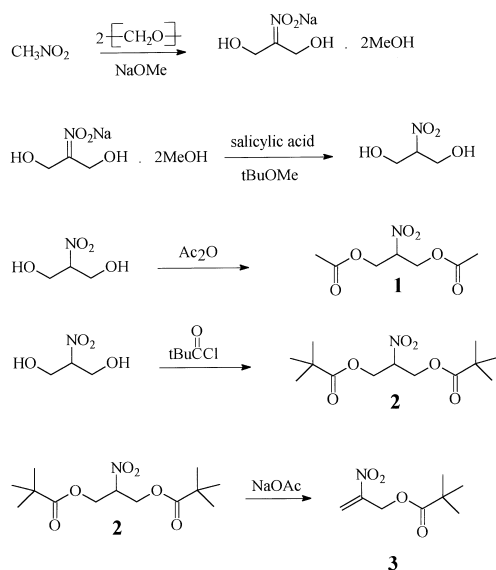
Scheme 1. Synthesis of 2-nitroalkyl starch ethers (etherification can also occur on O-3 and O-6).

precursors for cross-linked 2-aminoalkyl starch ethers, is also described.

2. Results and discussion

Syntheses of the cross-linking agents.—The synthesis of 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (**1**), 1,3-di-*O*-pivaloyl-2-nitro-1,3-propanediol (**2**) and 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**) is described by Seebach and Knochel [12]. The syntheses of **1** and **2** (Scheme 2) are straightforward, the yields are high and the procedure appears to be applicable for large scale reactions. The synthesis of **3** is achieved by adding a weak base (NaOAc) to **2**. Under these weakly alkaline conditions one molecule of pivalic acid splits off and a nitroalkene is formed. However, the yield of the selective multicoupling reagent is disappointing, the overall yield from formaldehyde, nitromethane and pivaloyl chloride being only about 25%.

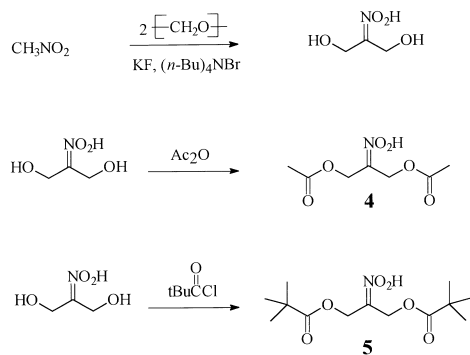
Also described in the literature is the synthesis of an intramolecularly stabilized nitronic acid, *aci*-2-nitro-1,3-propanediol [13]. High yields (73%) of this compound are obtained by reaction of nitromethane and paraformaldehyde with catalytic



Scheme 2. Synthesis of 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (**1**), 1,3-di-*O*-pivaloyl-2-nitro-1,3-propanediol (**2**), and 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**).

amounts of potassium fluoride and tetrabutylammonium bromide. Surprisingly, the *aci*-nitrocompounds 1,3-di-*O*-acetyl-*aci*-2-nitro-1,3-propanediol (**4**) and 1,3-di-*O*-pivaloyl-*aci*-2-nitro-1,3-propanediol (**5**) are isolated in moderate to good yields after acetylation with acetic anhydride or reaction with pivaloyl chloride (Scheme 3). Attempts to synthesize 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**) from **5** were not successful.

The synthesis of α,ω -bis(β -nitroacetoxyalkanes), in which the β -nitroacetoxy functionalities are separated by a short hydrophobic chain, is complicated. To our knowledge no satisfactory route for the synthesis of α,ω -dinitroalkanes has been described in the literature. Low to moderate yields are obtained for the reaction of α,ω -diiodoalkanes with silver nitrite [14]. Further reaction of the



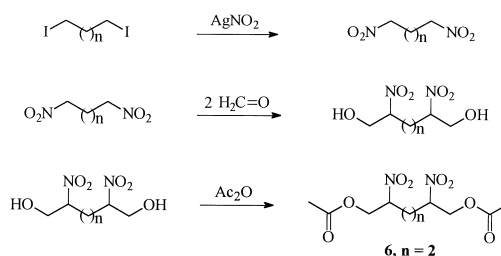
Scheme 3. Synthesis of 1,3-diacetyl-*aci*-2-nitro-1,3-propanediol (**4**) and 1,3-dipivaloyl-*aci*-2-nitro-1,3-propanediol (**5**).

α,ω -dinitroalkanes with formaldehyde (Henry reaction) followed by acetylation proceeds in good yields for the synthesis of 1,6-di-*O*-acetyl-2,5-dinitro-1,6-hexanediol (**6**; Scheme 4) [15].

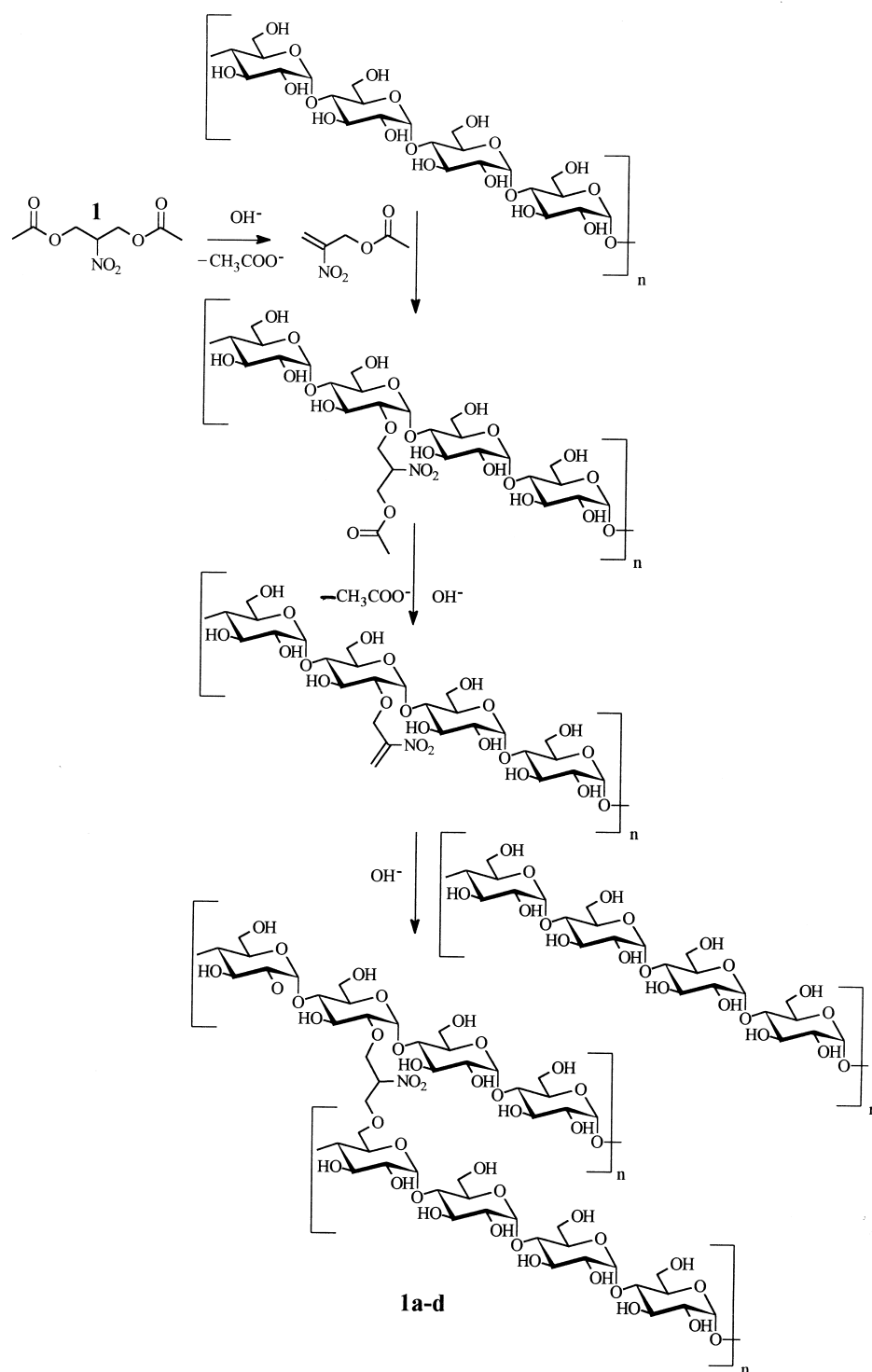
Unfortunately 1,3-dinitropropane is not stable under the reaction conditions required for the Henry reaction with formaldehyde. Due to the alkaline conditions a reverse aldol condensation probably takes place in which nitromethane and nitroethene are formed. Nitroethene is known to be very sensitive to anionic polymerisation.

Cross-linking of granular starch.—The anticipated pathway for cross-linking of granular starch suspensions with 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (**1**) is shown in Scheme 5. Under alkaline conditions acetic acid splits off and 2-nitro-3-*O*-acetyl-1-propene-3-ol is formed. It is expected that the *aci*-compound **4** reacts according to a similar pathway. If the pivaloyl derivatives **2** and **5** are used, pivalic acid splits off, which results in the formation of 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**). After a Michael addition of the corresponding nitroalkene with starch another molecule of acetic acid or pivalic acid is split off and a 2-nitro-3-propene starch ether is formed. A second Michael addition with starch completes the cross-link. It should be noted that the cross-link can be intramolecular within the glucose monomer, intramolecular within the same starch chain and intermolecular between different starch chains. The rheology of gelatinized cross-linked starches is predominantly influenced by intermolecular cross-links. Reaction may take place with any of the free hydroxyl groups.

The cross-linking of starch with α,ω -bis(β -nitroacetoxyalkanes) (Scheme 6) is comparable to the synthesis of 2-nitroalkyl starch ethers. Formation of a (di)nitroalkene and Michael additions of both sides of the molecule result in a cross-link within the starch granule. In this way a (short) hydrophobic chain is introduced into the starch matrix.



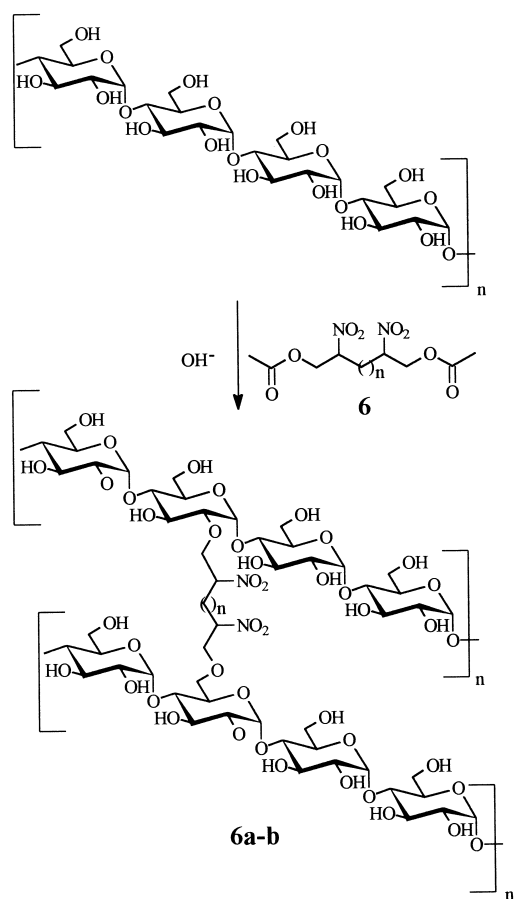
Scheme 4. Synthesis of α,ω -bis(β -nitroacetoxyalkanes).



Scheme 5. Example of the cross-linking reaction; reaction sequence of 1,3-di-O-acetyl-2-nitro-1,3-propanediol with starch.

Viscosity profiles of cross-linked starches.—Information about the efficiency of the cross-linking can be obtained from viscosity profiles (physical analysis). When suspensions of starch granules are heated above a certain temperature, water penetrates into the granules and weakens the

hydrogen bonds in starch segments. The viscosity of the suspension rises but when shear is imposed (by stirring) the integrity of the swollen starch granules is lost and the viscosity of the solution decreases. Amylose, followed by amylopectin, leaks out of the “ghosts” and goes into solution.



Scheme 6. Synthesis of distarch α,ω -bis(β -nitro)alkanediol using (1,6-di-*O*-acetyl-2,5-dinitro-1,6-hexanediol (**6**, $n = 2$).

After cooling down, the viscosity of the starch paste increases again. This gelatinization behaviour can be followed with a Rapid Visco Analyser (RVA), which measures the viscosity of the starch suspension while stirring the suspension with a revolving cup driven at constant speed [16]. The suspension passes through a certain temperature profile (1 min, 30 °C; 5.4 min, 30 °C→95 °C; 2.5 min, 95 °C; 3.7 min, 95 °C→50 °C; 2.1 min, 50 °C).

The gelatinization behaviour of cross-linked starches depends on the extent of cross-linking [17–20]. If the number of cross-links is high enough (≥ 1 cross-link per 100 glucose monomers) and the starch granules are heated above their gelatinization temperature, the crystallinity is lost but the granule shape remains intact. No increase in viscosity is observed. At low levels of cross-linking the viscosity of the starch solution is altered. At approximately 1 cross-link per 500 glucose monomers the maximum and the end viscosity are lower

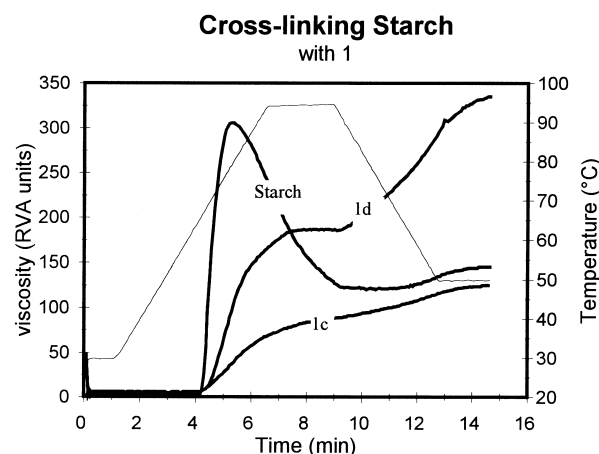


Fig. 1. RVA curves of starch and distarch 2-nitropropanediol (5% suspension; **1c**, $ds_{\max} = 0.0019$; **1d**, $ds_{\max} = 0.00098$).

than for gelatinized starch. At approximately 1 cross-link per 1000–1500 glucose monomers the maximum viscosity is lower in many cases but the end viscosity is higher than native gelatinized starch [4]. The same trend was observed for viscosity profiles (Fig. 1) of distarch 2-nitropropanediol (**1c**, $ds_{\max} = 0.0019$; **1d**, $ds_{\max} = 0.00098$ ¹) cross-linked with 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol. With larger numbers of cross-links ($ds_{\max} = 0.019$) no increase in viscosity was observed during the temperature programme. The granule form remains intact but the expected loss of crystallinity was confirmed with polarization microscopy.

The RVA curves for the products of the synthesis of distarch 2-nitropropanediol with compounds **1–5** (**1c**, **2a**, **4a**, **5a**, $ds_{\max} = 0.0019$; **3a**, $ds_{\max} = 0.0016$), distarch glycerol (epichlorohydrin **7b**, $ds_{\max} = 0.0022$) and native potato starch (5% solution) are shown in Fig. 2. One can readily conclude that cross-linking of granular starch with compounds **1** (1,3-di-*O*-acetyl-2-nitro-1,3-propanediol), **2** (1,3-di-*O*-pivaloyl-2-nitro-1,3-propanediol) and **4** (1,3-di-*O*-acetyl-*aci*-2-nitro-1,3-propanediol) leads to a lower viscosity profile than cross-linking with epichlorohydrin (with the same degree of substitution). Cross-linking with compound **5** (1,3-di-*O*-pivaloyl-*aci*-2-nitro-1,3-propanediol) results in products with almost similar peak and end viscosities (after the temperature programme) as epichlorohydrin (**7b**). Under the alkaline reaction conditions anionic polymerization of the

¹ ds_{\max} = the maximum number of cross-links per mole glucose monomer of the cross-linked starch.

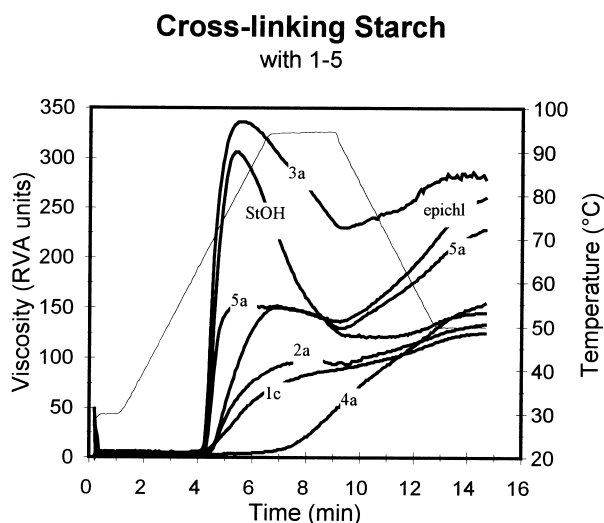


Fig. 2. RVA curves of distarch 2-nitropropanediol (5% suspension; synthesized with compounds 1–5 (1c–2a, 4a–5a, $ds_{\max} = 0.0019$; 3a, $ds_{\max} = 0.0016$), distarch glycerol (epichlorohydrin. 7b, $ds_{\max} = 0.0022$), and native starch.

nitroalkene 3 (2-nitro-3-*O*-pivaloyl-1-propene-3-ol) probably leads to less efficient cross-linking for 3a.

The viscosity profiles of granular starch cross-linked with the α,ω -bis(β -nitroacetoxyalkane) 1,6-di-*O*-acetyl-2,5-dinitro-1,6-hexanediol also fall in the range mentioned above. For relatively highly substituted distarch 2,5-dinitro-1,6-hexanediol (6a, $ds_{\max} = 0.0065$) no increase in viscosity was observed during the temperature programme. Lower substituted distarch 2,5-dinitro-1,6-hexanediol (6b, $ds_{\max} = 0.0011$) gave a higher end viscosity than granular starch after gelatinization (Fig. 3).

Swelling properties of cross-linked starches.—Although gelatinized starches are often referred to as solutions, these systems in fact consist of parti-

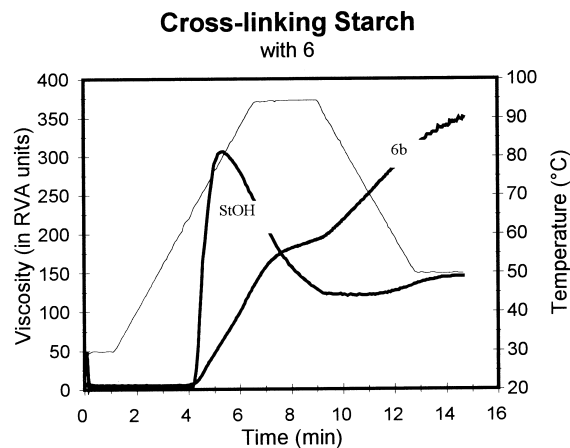


Fig. 3. RVA curves of distarch 2,5-dinitro-1,6-hexanediol (6b, $ds_{\max} = 0.0011$) and starch.

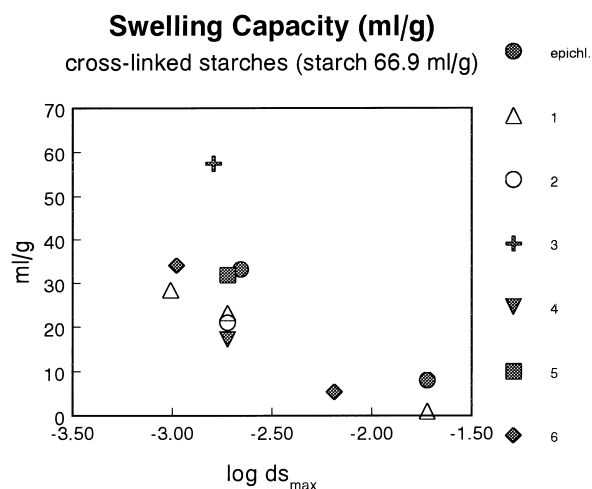


Fig. 4. Particle volume (swelling capacity) of starch cross-linked with bifunctional precursors of nitroalkenes.

cles [21]. After centrifugation a supernatant phase, which contains some soluble material, and a starch gel are obtained. The volume of the starch particles depends on the botanic source of the starch used [22]. As suspected, the cross-linking of starch has a drastic influence on the volume of the centrifuged starch particles. Due to the cross-links the aggregates are packed tighter and the volume of the gelatinized starch particles is reduced.

Comparing the particle volume of the centrifuged cross-linked starch at the same degree of substitution gives some information about the efficiency of the cross-linking with different cross-linking agents. However, it should be taken into account that topochemical aspects (distribution across the granule, amylose/amylopectin distribution) can also influence the swelling capacities of these aggregates. The particle volumes (swelling properties) of the cross-linked starches synthesized are shown in Fig. 4.

As expected, within one series using the same cross-linking agent, the swelling capacity of the cross-linked starches is decreased at higher degrees of substitution. The swelling capacities of starch cross-linked with bifunctional precursors for nitroalkenes (1, 2 and 4–6) are lower than starch cross-linked with commercially used epichlorohydrin. This means not only that the reaction rate of cross-linking is considerably higher than in the case of cross-linking with epichlorohydrin, but probably also that the efficiency of the cross-linking with 1, 2 and 4–6 is higher. The results obtained are fairly consistent with the RVA curves as shown in Figs. 1 and 2. Low end viscosities and swelling capacities were obtained for 1c,

2a, **4a** and **6b**, moderate end viscosity and swelling capacity was obtained for **5a**, and a high end viscosity and swelling capacity was obtained for **3a**. It can therefore be concluded that a novel series of easily synthesized cross-linkers for starch (and other polysaccharides), based on Michael additions to (di)nitroalkenes, has been developed.

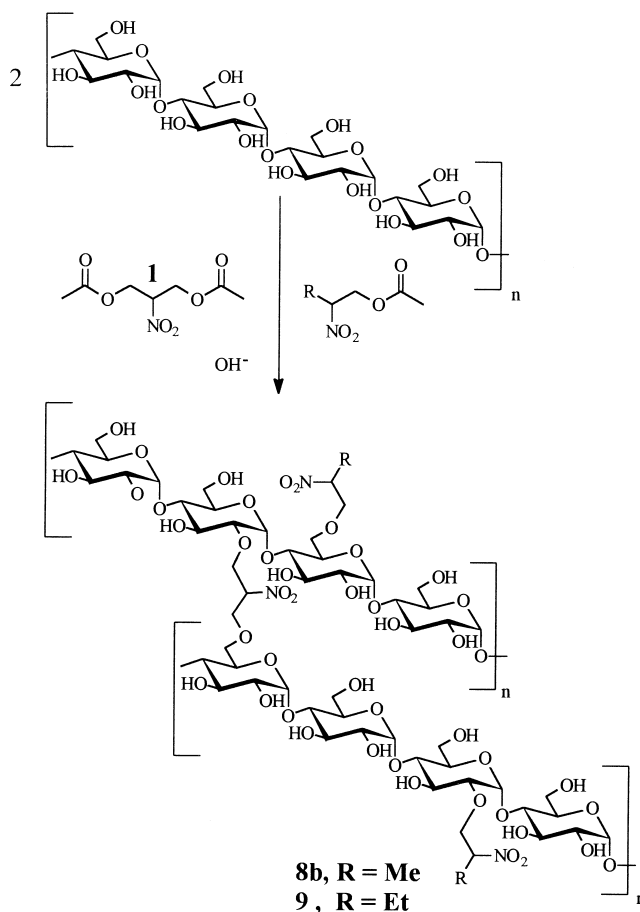
Chemical characterization of cross-linked starches.—Chemical analysis of cross-linked starches is often complicated due to the low degrees of substitution [23–26]. However, the ratio between the real cross-linking degree and mono-functionalization and topochemical aspects are of importance for the physical behaviour of these compounds. For the cross-linking of starch with epichlorohydrin the amount of mono-functionalization (the monoether glycerol bond) has been established by oxidation of the modified starch with NaIO_4 and subsequent determination of formaldehyde [19]. Gel permeation chromatograms of gelatinized distarch glycerol (low degrees of cross-linking) are in agreement with cross-links between amylose and amylopectin in the amorphous regions of the starch granule [27].

The efficiency of the reaction of starch with 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (**1a**) can be determined from the incorporation of nitrogen and was found to be 85%. The infrared absorbance of the nitro functionality (1552 cm^{-1}) is in agreement with values obtained for 2-nitroalkyl starch ethers. We have also attempted to determine the amount of monofunctionalization, side reactions, and the distribution over the different hydroxyl groups by synthesizing 1,3-di-*O*-acetyl-*aci*-2-nitro-1,3-propanediol-2- ^{13}C . After reaction with starch, the labeled distarch 2-nitropropanediol-2- ^{13}C (**4b**, $\text{ds}_{\text{max}} = 0.020$) was gelatinized and the swollen “granules” were treated with an enzyme cocktail of α -amylase (EC 3.2.1.1), amyloglucosidase (EC 3.2.1.3) and isoamylase (EC 3.2.1.68). Normally this procedure yields a mixture of glucose and substituted oligomers which can subsequently be separated by GPC and analyzed with ^{13}C NMR spectroscopy. Unfortunately, the cross-linked material was not sufficiently accessible to the enzymes for hydrolysis to occur.

Synthesis of 2-nitroalkyl distarch 2-nitropropanediol ethers.—Cross-linking of starch is often used in combination with another chemical modification [28–31]. It has been demonstrated that aminoalkyl cross-linked starches have potential for application as ion-exchange resins and

these compounds are excellent materials for the complexation of heavy metals ions [32–35]. In principle, primary or secondary aminoalkyl cross-linked starches (or other polysaccharides) are interesting materials for the immobilization of enzymes [36]. Such compounds can be prepared readily in a two-step (or possibly a one-step) procedure: synthesis of 2-nitroalkyl distarch 2-nitropropanediol followed by reduction of the nitro functionalities.

2-Nitroalkyl distarch 2-nitropropanediol (Scheme 7) is synthesized by nitroalkylation of distarch 2-nitropropanediol with 2-nitropropyl acetate (**8a**). The cross-linking and nitroalkylation of granular starch can also be accomplished in a one pot synthesis. In this way 2-nitropropyl distarch 2-nitropropanediol (**8b**, **10**) and 2-nitrobutyl distarch 2-nitropropanediol (**9**, **11**) were synthesized by addition of 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol/2-nitroalkyl acetate (**8b**, **9**) or 1,3-di-*O*-acetyl-*aci*-2-nitro-1,3-propanediol/2-nitroalkyl acetate (**10**, **11**).



Scheme 7. Structure of 2-nitroalkyl distarch 2-nitropropanediol ethers.

The efficiency of the nitroalkylation is moderate (for the 2-nitrobutyl ether) to high (for the 2-nitropropyl ether). The nitroalkyl functionality decreases the gelatinization temperature of the cross-linked nitroalkyl starch ethers (native starch $T_{\text{gel}} = 59.3^\circ\text{C}$). Owing to the cross-links no viscosity is observed for the gelatinized cross-linked 2-nitroalkyl starch derivatives (Table 1).

The reduction of these 2-nitroalkyl cross-linked starch ethers to the corresponding 2-aminoalkyl cross-linked starch derivatives is currently under investigation.

3. Experimental

General methods.—1,3-Di-*O*-acetyl-2-nitro-1,3-propanediol (**1**) [12], 1,3-di-*O*-pivaloyl-2-nitro-1,3-propanediol (**2**) [12], 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**) [12], 1,6-di-*O*-acetyl-2,5-dinitro-1,6-hexanediol (**6**) [15], *aci*-2-nitro-1,3-propanediol [13], *aci*-2-nitro-1,3-propanediol-2- ^{13}C [13], 2-nitropropyl acetate [37] and 2-nitrobutyl acetate [36] were prepared according to literature procedures. Formaldehyde (37% solution), acetic anhydride, 1-nitropropane (95%), NaOH pellets, KF and tetrabutylammonium bromide were purchased from Merck. Nitromethane (96%), nitromethane- ^{13}C (99% labeled), nitroethane (96%), salicylic acid, AgNO_2 , sodium methoxide (30% solution), epichlorohydrin, pivaloyl chloride, and di-iodobutane were purchased from Aldrich. Blue dextran was purchased from Pharmacia Biotech. Potato starch was a gift from AVEBE (Foxhol, The Netherlands). ^1H and ^{13}C NMR spectra were recorded on a 300 MHz Varian VTR-300 spectrometer. FT-IR spectra were recorded on a Bio-Rad FTS 135 spectrometer. Differential scanning calorimetry was performed on a Perkin–Elmer

DSC7 PC Series apparatus (heating rate 5.0 K/min). Viscosity profiles were recorded on a Rapid Visco Analyser RVA-3d purchased from Newport Scientific. Spectrophotometric measurements were performed with a LKB Novaspec II from Biochrom. The pH-stat, a Titration Controller 1200 equipped with a Titronic T110 burette, was purchased from Schott Geräte. Nitrogen analyses were performed at the Analytical Department of AVEBE (Foxhol, The Netherlands). Molar substitution of the 2-nitroalkyl substituent was determined according to the formula $ms = 1.62N / \{14 - 0.01N(\text{Mwt}_{\text{alkyl subst}} - 1)\}$ (N = nitrogen content measured in %N and $\text{Mwt}_{\text{alkyl subst}}$ = molecular weight of the nitroalkyl substituent).

Synthesis of 1,3-di-*O*-acetyl-*aci*-2-nitro-1,3-propanediol (4**)².**—A drop of conc H_2SO_4 was added to a stirred solution of *aci*-2-nitro-1,3-propanediol (2.63 g, 21.7 mmol) and Ac_2O (4.45 g, 43.6 mmol). After 3 h, CH_2Cl_2 (20 mL) was added and the mixture was poured into sat NaHCO_3 (25 mL). After separation of the organic layer, the NaHCO_3 solution was washed with CH_2Cl_2 (20 mL) and the combined CH_2Cl_2 layers were dried over Na_2SO_4 . After filtration and concentration, the residue was distilled under reduced pressure (0.1 mm Hg, 200°C) and **4** was isolated (2.42 g, 55%). NMR (CDCl_3): ^1H , δ 4.54 (s, 4 H, CH_2O), 2.08 (s, 6 H, CH_3); ^{13}C , δ 169.51 (C=O), 88.01 (CNO_2), 60.49 (CH_2O), 20.43 (CH_3). FT-IR (KBr): 2968 (w), 2905 (w), 1825 (w), 1756 (s), 1559 (s), 1465 (m), 1438 (w), 1382 (s), 1369 (s), 1224 (s), 1126 (m), 1048 (s), 1016 (m), 959 (w), 900 (w), 840 (w), 737 (w), 666 (w) cm^{-1} .

Synthesis of 1,3-di-*O*-pivaloyl-*aci*-2-nitro-1,3-propanediol (5**).**—Pivaloyl chloride (10.01 g, 83 mmol) was added to a solution of *aci*-2-nitro-1,3-propanediol (3.25 g, 26.9 mmol) in CH_2Cl_2 (15 mL). After boiling under reflux for 20 h and concentration, the residue was dissolved in CH_2Cl_2 (15 mL) and washed with sat NaHCO_3 (3×10 mL) and H_2O (2×10 mL). The CH_2Cl_2 layer was dried (Na_2SO_4) and, after filtration and concentration, crude **5** (6.02 g) was isolated. Part of the residue (3.35 g) was distilled under reduced pressure (0.02 mm Hg, 150°C) and **5** (2.59 g, 60%) was isolated. NMR (CDCl_3): ^1H , δ 4.50 (s, 4 H, CH_2O), 1.15 (s, 18 H, CH_3); ^{13}C , δ 177.8 (C=O), 88.4 (CNO_2), 60.38 (CH_2), 38.90 (C-Piv), 26.64 (CH_3).

Table 1
Synthesis of 2-nitropropyl and 2-nitrobutyl distarch 2-nitropropanediol

Compound	ms (2nps) ^a	RE(%) ^b	T_{gel}^c	$ds_{\text{max}}(\text{crl})^d$	RVA ^e
8b	0.062	87	48.4	0.0034	0
9	0.046	65	50.3	0.0036	0
10	0.057	71	48.6	0.0033	0
11	0.043	61	54.7	0.0032	0

^a See Experimental.

^b Reaction efficiency (ms/ ms_{max}).

^c Gelatinization temperature (in $^\circ\text{C}$), determined with DSC (onset).

^d Maximum degree of cross-linking.

^e In RVA units.

² The synthesis of 1,3-di-*O*-acetyl-*aci*-2-nitro-1,3-propanediol-2- ^{13}C is similar to the synthesis of **4**.

FT-IR (KBr): 2976 (s), 2939 (s), 2912 (s), 2876 (s), 1743 (s), 1556 (s), 1481 (s), 1466 (s), 1399 (m), 1369 (m), 1352 (m), 1282 (s), 1135 (s), 1039 (m), 1014 (w), 999 (w), 985 (w), 944 (w), 922 (w), 865 (m), 845 (w), 813 (w), 798 (w), 784 (w), 767 (m), 665 (w) cm^{-1} .

Cross-linking of starch with 1,3-di-O-acetyl-2-nitro-1,3-propanediol (1a), $ds_{\text{max}} = 0.096$.—Native starch (100.01 g, 520 mmol) was suspended in H_2O (100 mL). The pH of the aqueous starch suspension was adjusted to 10.0 with 1 M NaOH. 1,3-Di-O-acetyl-2-nitro-1,3-propanediol (**1**; 10.25 g, 50 mmol) was added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 16 h the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After filtration and washing with water the product was dried at 40 °C and distarch 2-nitropropanediol (**1a**; 95.54 g, dry substance = 88.44%, %N = 0.68%) was isolated. FT-IR (KBr): 3453 (s), 2928 (m), 1658 (m), 1552 (m), 1164 (s), 988 (s), 857 (m), 763 (m), 573 (m) cm^{-1} .

Cross-linking of starch with 1,3-di-O-acetyl-2-nitro-1,3-propanediol (1b), $ds_{\text{max}} = 0.019$.—The same procedure was followed as for **1a** with starch (100.70 g, 520 mmol), H_2O (100 mL) and 1,3-di-O-acetyl-2-nitro-1,3-propanediol (2.05 g, 10.0 mmol). Isolated: 94.26 g distarch 2-nitropropanediol (**1b**; dry substance = 89.27%).

Cross-linking of starch with 1,3-di-O-acetyl-2-nitro-1,3-propanediol (1c), $ds_{\text{max}} = 0.0019$.—The same procedure was followed as for **1a** with starch (250.02 g, 1.30 mol), H_2O (250 mL) and 1,3-di-O-acetyl-2-nitro-1,3-propanediol (0.51 g, 2.49 mmol). Isolated: 222.20 g distarch 2-nitropropanediol (**1c**; dry substance = 91.65%).

Cross-linking of starch with 1,3-di-O-acetyl-2-nitro-1,3-propanediol (1d), $ds_{\text{max}} = 0.00098$.—The same procedure was followed as for **1a** with starch (250.88 g, 1.30 mol), H_2O (250 mL) and 1,3-di-O-acetyl-2-nitro-1,3-propanediol (0.26 g, 12.7 mmol). Isolated: 229.74 g distarch 2-nitropropanediol (**1d**; dry substance = 91.45%).

Cross-linking of starch with 1,3-di-O-pivaloyl-2-nitro-1,3-propanediol (2a), $ds_{\text{max}} = 0.0019$.—The same procedure was followed as for **1a** with starch (100.33 g, 520 mmol), H_2O (100 mL) and 1,3-di-O-pivaloyl-2-nitro-1,3-propanediol (0.29 g, 1.00 mmol). After stirring for 48 h distarch 2-nitropropanediol (**2a**; 91.08 g, dry substance = 90.94%) was isolated.

Cross-linking of starch with 2-nitro-3-O-pivaloyl-1-propene-3-ol (3a), $ds_{\text{max}} = 0.0016$.—The same procedure was followed as for **1a** with starch (100.04 g, 520 mmol), H_2O (100 mL) and 2-nitro-3-O-pivaloyl-1-propene-3-ol (0.16 g, 0.86 mmol). After stirring for 16 h distarch 2-nitropropanediol (**3a**; 90.28 g, dry substance = 91.11%) was isolated.

Cross-linking of starch with 1,3-di-O-acetyl-2-nitro-1,3-propanediol (4a), $ds_{\text{max}} = 0.0019$.—The same procedure was followed as for **1a** with starch (100.02 g, 520 mmol), H_2O (100 mL) and 1,3-di-O-acetyl-2-nitro-1,3-propanediol (0.20 g, 1.0 mmol). After stirring for 16 h distarch 2-nitropropanediol (**4a**; 89.60 g, dry substance = 91.73%) was isolated.

Cross-linking of starch with 1,3-di-O-acetyl-2-nitro-1,3-propanediol-2- ^{13}C (4b), $ds_{\text{max}} = 0.020$.—The same procedure was followed as for **1a** with starch (10.01 g, 52.0 mmol), H_2O (12 mL) and 1,3-di-O-acetyl-2-nitro-1,3-propanediol-2- ^{13}C (0.21 g, 1.02 mmol in 1 mL 2-propanol). After stirring for 16 h distarch 2-nitropropanediol-2- ^{13}C (**4b**; 9.13 g) was isolated.

Cross-linking of starch with 1,3-di-O-pivaloyl-2-nitro-1,3-propanediol (5a), $ds_{\text{max}} = 0.0019$.—The same procedure was followed as for **1a** with starch (100.02 g, 520 mmol), H_2O (100 mL) and 1,3-di-O-pivaloyl-2-nitro-1,3-propane (0.29 g, 1.0 mmol). After stirring for 48 h distarch 2-nitropropanediol (**5a**; 90.16 g, dry substance = 91.16%) was isolated.

Cross-linking of starch with 1,6-di-O-acetyl-2,5-dinitro-1,6-hexanediol (6a), $ds_{\text{max}} = 0.0065$.—The same procedure was followed as for **1a** with starch (10.0 g, 52 mmol), H_2O (10 mL) and 1,6-di-O-acetyl-2,5-dinitro-1,6-hexanediol (0.10 g, 0.34 mmol). After stirring for 20 h distarch 2,5-dinitrohexanediol (**6a**; 9.99 g, dry substance = 83.78%, air dried) was isolated.

Cross-linking of starch with 1,6-di-O-acetyl-2,5-dinitro-1,6-hexanediol (6b), $ds_{\text{max}} = 0.0011$.—The same procedure was followed as for **1a** with starch (10.0 g, 52 mmol), H_2O (10 mL) and 1,6-di-O-acetyl-2,5-dinitro-1,6-hexanediol (0.016 g, 0.055 mmol). After stirring for 20 h distarch 2,5-dinitrohexanediol (**6b**; 9.98 g, dry substance = 81.19%, air dried) was isolated.

Cross-linking of starch with epichlorohydrin (7a), $ds_{\text{max}} = 0.019$.—Native starch (250.02 g, 1.30 mol) was suspended in H_2O (250 mL). The pH of the aqueous starch suspension was adjusted to 11.0 with 1 M NaOH and heated to 35 °C. Epichlorohydrin (2.28 g, 25 mmol) was added and after 48 h the suspension was neutralized with

0.5 M HCl. After filtration and washing with water the product was dried at 40 °C, and 235.35 g distarch glycerol (**7a**; dry substance = 86.09%) was isolated.

Cross-linking of starch with epichlorohydrin (7b), $ds_{max} = 0.0022$.—The same procedure was followed as for **7a** with starch (150.02 g, 0.78 mol), H₂O (150 mL) and epichlorohydrin (0.16 g, 1.73 mmol). After stirring for 48 h distarch glycerol (**7b**; 134.76 g, dry substance = 90.84%) was isolated.

Synthesis of 2-nitropropyl distarch 2-nitropropanediol (8a).—Cross-linked starch (**1a**; 25.00 g, 145 mmol) was suspended in H₂O (50 mL). The pH of the aqueous cross-linked starch suspension was adjusted to 10.0 with 1 M NaOH. 2-Nitropropyl acetate (10.27 g, 69.9 mmol) was added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 16 h the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After filtration and washing with water, the product was dried to air, and 26.50 g 2-nitropropyl distarch 2-nitropropanediol (**8a**; %N = 2.65%, dry substance not determined due to decomposition of the material) was isolated. FT-IR (KBr): 3448 (s), 2930 (s), 1655 (m), 1554 (s), 1459 (m), 1364 (m), 1164 (s), 1083 (s), 988 (s), 933 (m), 850 (m), 763 (m), 709 (m), 573 (m), 523 (m) cm⁻¹.

Synthesis of 2-nitropropyl distarch 2-nitropropanediol (with 1,3-di-O-acetyl-2-nitro-1,3-propanediol, one pot synthesis) (8b).—Native starch (49.96 g, 260 mmol) was suspended in H₂O (50 mL), which contained Na₂SO₄ (2.53 g, 17.8 mmol). The pH of the aqueous starch suspension was adjusted to 10.0 with 1 M NaOH. 1,3-Di-O-acetyl-2-nitro-1,3-propanediol (0.18 g, 0.88 mmol) and 2-nitropropyl acetate (2.72 g, 18.5 mmol) were added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 1 h the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After filtration and washing with water, the product was dried at 40 °C and 45.19 g 2-nitropropyl distarch 2-nitropropanediol (**8b**; dry substance = 92.65%, %N = 0.52%) was isolated. FT-IR (KBr): 3456 (s), 2928 (s), 1658 (m), 1550 (m), 1465 (m), 1164 (s), 1083 (m), 983 (s), 928 (m), 859 (m), 764 (m), 709 (m), 573 (m) cm⁻¹.

Synthesis of 2-nitrobutyl distarch 2-nitropropanediol (with 1,3-di-O-acetyl-2-nitro-1,3-propanediol, one pot synthesis) (9).—Native starch (50.10 g, 260 mmol) was suspended in H₂O (50 mL),

which contained Na₂SO₄ (2.52 g, 17.8 mmol). The pH of the aqueous starch suspension was adjusted to 10.0 with 1 M NaOH. 1,3-Di-O-acetyl-2-nitro-1,3-propanediol (0.19 g, 0.93 mmol) and 2-nitrobutyl acetate (3.00 g, 18.6 mmol) were added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 2 h the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After filtration and washing with water, the product was dried at 40 °C, and 45.39 g 2-nitrobutyl distarch 2-nitropropanediol (**9**; dry substance = 92.61%, %N = 0.39%) was isolated. FT-IR (KBr): 3451 (s), 2930 (s), 1654 (m), 1546 (m), 1464 (m), 1163 (s), 986 (s), 928 (m), 859 (m), 764 (m), 709 (m), 573 (m) cm⁻¹.

Synthesis of 2-nitropropyl distarch 2-nitropropanediol (with 1,3-di-O-acetyl-aci-2-nitro-1,3-propanediol, one pot synthesis) (10).—Native starch (100.01 g, 520 mmol) was suspended in H₂O (100 mL), which contained Na₂SO₄ (5.04 g, 35.6 mmol). The pH of the aqueous starch suspension was adjusted to 10.0 with 1 M NaOH. 1,3-Di-O-acetyl-aci-2-nitro-1,3-propanediol (0.35 g, 1.70 mmol) and 2-nitropropyl acetate (5.45 g, 37.1 mmol) were added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 3 h the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After filtration and washing with water, the product was dried at 40 °C, and 93.54 g 2-nitropropyl distarch 2-nitropropanediol (**10**; dry substance = 91.49%, %N = 0.48%) was isolated. FT-IR (KBr): 3434 (s), 2930 (s), 1654 (m), 1548 (m), 1464 (m), 1164 (s), 986 (s), 859 (m), 764 (m), 709 (m) cm⁻¹.

Synthesis of 2-nitrobutyl distarch 2-nitropropanediol (with 1,3-di-O-acetyl-aci-2-nitro-1,3-propanediol, one pot synthesis) (11).—Native starch (75.03 g, 389 mmol) was suspended in H₂O (75 mL), which contained Na₂SO₄ (3.77 g, 26.6 mmol). The pH of the aqueous starch suspension was adjusted to 10.0 with 1 M NaOH. 1,3-Di-O-acetyl-aci-2-nitro-1,3-propanediol (0.26 g, 1.26 mmol) and 2-nitrobutyl acetate (4.42 g, 27.5 mmol) were added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 3 h the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After filtration and washing with water, the product was dried at 40 °C and 68.85 g 2-nitrobutyl distarch 2-nitropropanediol (**11**; dry substance

=91.08%, %N=0.36%) was isolated. FT-IR (KBr): 3468 (s), 2929 (s), 1653 (m), 1545 (m), 1464 (m), 1163 (s), 985 (s), 928 (m), 859 (m), 764 (m), 708 (m), 573 (m) cm^{-1} .

Determination of the swelling capacity.—The swelling capacities of the cross-linked starches **1–7b** were determined by a slightly modified procedure of Evans [25]. 2.00 g Blue dextran (0.3%) was added to a suspension of cross-linked starch (40–400 mg) in 8.00 mL H_2O . The closed tubes were rotated for 45 min at 90–95 °C in a ventilating fan. After cooling down, the tubes were centrifuged [15 min, 3000 rpm (swing out rotor)]. The supernatant liquid (1.5 mL) was filtered and the absorbance of the initial and the obtained supernatant solutions were measured in a spectrophotometer at 620 nm. The swelling capacity (SC) was determined by the formula: $\text{SC} = 10 \cdot (A - A_0/A) / w \cdot d_s$ mL/g (A = extinction cross-linked starch, A_0 = extinction blue dextran (control), w = weight cross-linked starch, d_s = dry substance cross-linked starch).

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